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The associations between serum leptin, adiponectin and intercellular adhesion molecule-1 in hypercholesterolemic patients*

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Abstract

We examined the associations between adiponectin or leptin and serum ICAM-1 levels in seventy-six hypercholesterolemic patients (mean age 59 yrs, 25 males and 51 females, LDL-cholesterol ≥ 130 mg/dL at screening). Blood lipid profiles and HOMA-IR derived from fasting glucose and insulin concentrations were determined. Serum levels of adiponectin, leptin and ICAM-1 were analyzed using ELISA. The results showed that serum levels of leptin were positively associated with serum levels of ICAM-1 independent of age, sex and BMI ($r = 0.392$, $p < 0.001$). Serum levels of adiponectin were negatively associated with serum levels of ICAM-1 independent of age, sex and BMI ($r = -0.343$, $p < 0.005$). Stepwise multiple linear regression analysis showed that serum leptin was an independent factor to be associated with serum ICAM-1 levels after adjusting for age, sex, BMI, alcohol intake, smoking status, blood lipids such as total cholesterol, triglyceride, HDL cholesterol and LDL cholesterol and HOMA-IR ($p < 0.001$). With respect to adiponectin, its association with serum ICAM-1 was attenuated but still significant when further adjustments were made for age, sex, BMI, alcohol intake, smoking status, blood lipids such as total cholesterol, triglyceride, HDL cholesterol and LDL cholesterol and HOMA-IR ($p < 0.005$). In conclusion, this study suggests that adiponectin and leptin are associated with endothelial derived inflammation.

Key Words: Leptin, adiponectin, ICAM-1, adipokine, hypercholesterolemia

Introduction

Endothelial dysfunction is a collective term that incorporates a number of changes that the endothelium undergoes during atherogenesis, i.e. loss of anticoagulant properties, increased expression of cellular adhesion molecules and increased vascular tone due to loss of bioavailability of vasodilatory endothelial nitric oxide (Cines *et al.*, 1998; van Haelst *et al.*, 2003). In particular, current evidence supports a central role for inflammation in all phases of the atherosclerotic process. Inflammatory processes promote the leukocytes penetrate into the intima and in the arterial wall in early stage. Through the processes, the blood-derived inflammatory cells participate in and perpetuate a local inflammatory response and also contribute decisively to precipitating acute thrombotic complications of atheroma (Libby *et al.*, 2002; Libby, 2002).

The endothelial intercellular adhesion molecule-1 (ICAM-1), a member of the immunoglobulin superfamily of cellular adhesion molecules, plays an important role in the initiation of the inflammatory process (Hopkins *et al.*, 2004). ICAM-1 interacts with adhesion molecules on leukocytes as a first step toward migration of the leukocytes into the arterial intima; thus, ICAM-1

plays a key role in the recruitment of immune cells during the development of atherosclerotic plaque (Kevil *et al.*, 2001).

It has been reported on the involvement of adipokines, which are proteins produced mainly by adipocytes, in proatherosclerotic process, linking obesity with obesity associated complications (Ahima, 2001; Chandran *et al.*, 2003). Adiponectin, one of the most abundant adipose tissue-specific adipokines, is reduced in obesity (Berg *et al.*, 2001; Okamoto *et al.*, 2002) and metabolic syndrome (Hu *et al.*, 1996). Furthermore, adiponectin attenuates the endothelial inflammatory response *in vitro*, and its concentration is decreased in patients with coronary artery disease (Yamauchi *et al.*, 2003). Adiponectin has been found to inhibit the expression of the adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), E-selectin and ICAM-1. Therefore, it interferes with monocyte adherence to endothelial cells and their subsequent migration to the subendothelial space, one of the initial events in the development of atherosclerosis (Ouchi *et al.*, 1999). Leptin is predominantly expressed by adipocytes, and its plasma levels correlated well with the body fat mass (Peelman *et al.*, 2004), fat accumulation, and insulin resistance (de Courten *et al.*, 1997; Zimmet *et al.*, 1996). Leptin possesses procoagulant and antifibrinolytic properties, and it

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promotes thrombus and atheroma formation, probably through the leptin receptors by promoting vascular inflammation, proliferation and calcification (Kougias *et al.*, 2005).

It has been reported that decreased serum adiponectin and increased leptin levels are found in subjects with familial combined hyperlipidemia characterized by increased levels of total cholesterol, triglycerides and/or apolipoprotein B (van der Vleuten *et al.*, 2005; van der Vleuten *et al.*, 2006). Levels of ICAM-1 were significantly increased in hypercholesterolemic patients (Hackman *et al.*, 1996). However information on associations among adipokines and endothelial function as expressed as ICAM-1 is still limited in hypercholesterolemic subjects. Therefore, the purpose of this study was to investigate relationship between adiponectin or leptin and serum ICAM-1 levels in hypercholesterolemic patients.

Subjects and Methods

Subjects

Seventy-six hypercholesterolemic patients (20-75 years old, 25 males and 51 females; low-density lipoprotein cholesterol [LDL-C] ≥ 130 mg/dL at screening) participated in the present study. Subjects who were taking hypolipidemic medication, antioxidative vitamins, or who had been diagnosed with type 2 diabetes mellitus, thyroid disorders, liver or renal dysfunction were excluded. Body weight and height were measured and BMI was calculated. Venous blood samples were collected from the forearm in EDTA-treated and plain tubes after a fasting period. The tubes were immediately covered with aluminum foil and placed on ice until they arrived at the analytical laboratory, where they were stored at -70°C . All patients gave written informed consent, and the institutional review board at the Yonsei University Medical Center approved the study protocol. We assessed cigarette smoking history using questions about current or past use of cigarettes and ex-smoker who reported quitting smoking 1 y before the start of study. Questions about alcohol intake assessed the frequency of drinking per week and the amount of alcohol consumed.

Serum lipid profiles

Serum cholesterol, LDL-C, and high-density lipoprotein cholesterol (HDL-C) were measured with commercially available kits (Choongwae, Seoul, Korea) by enzymatic methods. Serum triglyceride levels were analyzed using a total glycerol test kit (Roche, Basel, Switzerland). All determinants were done on a Hitachi 747 autoanalyzer (Hitachi, Tokyo, Japan).

Serum glucose, insulin, and homeostasis model assessment

Fasting serum glucose concentrations were measured by the

glucose oxidase method using a Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA). Insulin was measured by radioimmunoassay with commercial kits from Immuno Nucleo (Stillwater, MN). We calculated the homeostasis model assessment of insulin resistance (HOMA-IR) using the equation: $\text{HOMA-IR} = \text{fasting insulin } (\mu\text{U/mL}) \times \text{glucose (mmol/L)} / 22.5$ (Mathews *et al.*, 1985).

Serum sICAM-1, adiponectin and leptin

Serum sICAM-1 (Human ICAM-1, R&D Systems, Minneapolis, MN) and adiponectin levels (Human Adiponectin Enzyme-Linked Immunosorbent Assay Kit, Biovendor, BRNO, Czech Republic) were measured by enzyme immunoassay. The resultant color reaction was read with a Victor² measuring A450. A Packard Cobra II 5005 γ -Counter with Human Leptin Radioimmunoassay Kit (Linco, Research, St Charles, MO) was used to measure leptin concentrations.

Statistical analysis

The SPSS 12.0 software package (SPSS, Chicago, IL) was used for statistical analysis. Data were presented as mean \pm SD. Each variable was examined for normal distribution, and abnormally distributed variables were log-transformed. We used the Pearson correlation coefficient to evaluate relationships between variables. A stepwise multiple regression analysis was used to identify the factors which were associated with serum ICAM-1 levels. P values of less than .05 were considered statistically significant.

Results

Mean age and body mass index (BMI) of total subjects were 59.3 ± 9.3 years and 24.5 ± 2.9 kg/m², respectively (Male: Female = 25:51).

Table 1 presented the serum concentrations of serum lipids, leptin, adiponectin and ICAM-1 and calculated HOMA-IR of

Table 1. Characteristics of hypercholesterolemic subjects

	n = 76
Age (years)	59.3 \pm 9.3
Body mass index (kg/m ²)	24.5 \pm 2.9
Triglyceride (mg/dL)	172.1 \pm 83.2
Total cholesterol (mg/dL)	234.8 \pm 27.9
LDL-cholesterol (mg/dL)	149.4 \pm 21.5
HDL-cholesterol (mg/dL)	42.4 \pm 11.6
Fasting glucose (mg/dL)	86.1 \pm 8.7
Fasting insulin (μ U/ml)	8.3 \pm 4.1
HOMA-IR index	1.8 \pm 0.9
Leptin (ng/mL)	3.7 \pm 3.6
Adiponectin (μ g/mL)	5.9 \pm 3.9
ICAM-1 (ng/mL)	310.9 \pm 149.2

Means \pm S.D.

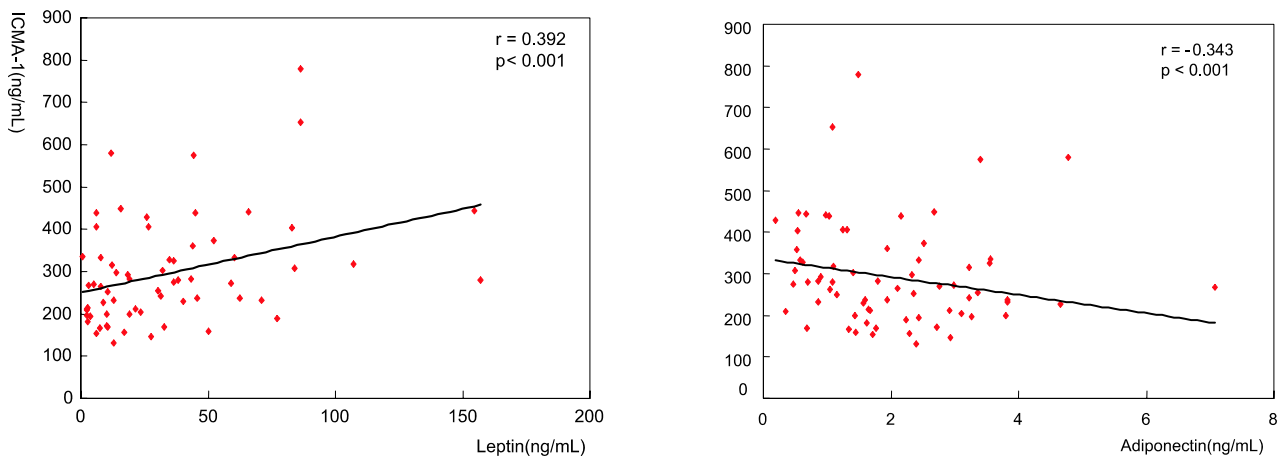


Fig. 1. Correlations between leptin or adiponectin and Serum ICAM-1 levels of the subjects

Table 2. Stepwise multiple regression analyses to identify factors influencing serum ICAM-1

Dependent variable	Model	Independent Variable	Adjusted β -coefficients	p-value	R	p-value
ICAM-1	1 step	Leptin	0.375	< 0.001	0.375	< 0.001
	2 step	Leptin	0.417	< 0.001	0.535	< 0.001
		Age	0.315	0.006		

Included independent variables: age, gender, BMI, alcohol intake, smoking status, blood lipids, HOMA-R, adiponectin and leptin.

total subjects. We examined correlations between serum ICAM-1 and two adipokine levels such as leptin and adiponectin. The results showed that serum levels of leptin were positively associated with serum levels of ICAM-1 independent of age, sex and BMI ($r = 0.392$, $p < 0.001$, Fig. 1). Serum levels of adiponectin were negatively associated with serum levels of ICAM-1 independent of age, sex and BMI ($r = -0.343$, $p < 0.005$, Fig. 1).

Stepwise multiple linear regression analysis was performed to examine the associations between levels of 2 adipokines and levels of ICAM-1. Serum leptin was an independent factor to be associated with serum ICAM-1 levels after adjusting for age, sex, BMI, alcohol intake, smoking status, blood lipids such as total cholesterol, triglyceride, HDL cholesterol and LDL cholesterol and HOMA-IR ($p < 0.001$). With respect to adiponectin, its association with serum ICAM-1 was attenuated but still significant when further adjustments were made for age, sex, BMI, alcohol intake, smoking status, blood lipids such as total cholesterol, triglyceride, HDL cholesterol and LDL cholesterol and HOMA-IR ($p < 0.005$). However, the association of the levels of adiponectin with the levels of ICAM-1 was no longer significant in a model including levels of leptin (Table 2).

Discussion

Circulating soluble ICAM-1 (sICAM-1) is a biochemical marker associated with atherosclerotic progression and other

inflammatory disease processes (van de Stolpe & van der Saag, 1996; Witkowska, 2005). Serum sICAM-1 levels have been found to be significantly elevated in patients with multiple risk factors for atherosclerosis, with coronary heart disease and myocardial infarction compared with matched control (Glowinska *et al.*, 2005; Ikata *et al.*, 2000; Zeitler *et al.*, 1997). Obesity is known to be one of the major risk factors for atherosclerosis. The obese state is characterized by what has been called low-grade systemic inflammation (Berg & Scherer, 2005). Compared with lean subjects, inflammatory markers, such as C-reactive protein (CRP), IL-6 and sICAM-1 are increased in obese individuals (Fantuzzi, 2005; Ziccardi *et al.*, 2002). Adipokines, such as adiponectin and leptin, of which secretory levels in blood are altered with excess adiposity, profoundly influence inflammatory process and thus may play important role in the atherosclerotic process (Lau, 2005; Lyon, 2003).

In the present study, we investigated the relationship between adiponectin or leptin and serum ICAM-1 levels in hypercholesterolemic patients. The concentrations of adiponectin and ICAM-1 in our subjects were 5.9 ± 3.9 ng/mL and 310.9 ± 149.2 ng/mL, respectively, which showed similar levels of 6.0 ± 1.4 ng/mL (Sonmez *et al.*, 2006) and 314 ± 36 ng/mL (Hackman *et al.*, 1996) in patients with dyslipidemia. We observed that serum adiponectin was negatively correlated with serum ICAM-1 level, whereas serum leptin showed significantly positive relation with serum ICAM-1 in hypercholesterolemic patients. These results are consistent with the previous human studies demonstrating the negative correlation between plasma adiponectin and ICAM-1 and positive correlation between plasma leptin and ICAM-1 (Kent *et al.*, 2004). Karaduman *et al.* (2006) reported that ICAM-1 levels were negatively correlated with adiponectin levels from human coronary atherosclerotic plaque.

Several *in vitro* and animal studies have indicated that adiponectin possesses anti-inflammatory properties. Physiological concentration of adiponectin reduced monocyte cell adhesion suppressing by TNF- α or resistin mediated mRNA expressions

of ICAM-1 in human aortic vascular endothelial cells (Kawanami *et al.*, 2004; Ouchi, 1999). Over-expression of adiponectin attenuated endothelial inflammatory response, in part by down-regulating adhesion molecules in apolipoprotein E-deficient mice (Okamoto *et al.*, 2002). From the literature, it is well known that leptin is an independent risk factor for cardiovascular diseases (CVD). Plasma leptin levels positively predicted cardiovascular events even after adjusting for traditional risk factor, BMI, and plasma C-reactive peptide (CRP) levels in a case-control study nested within the WOSCOPS clinical trial (Wallace *et al.*, 2001). Van der Vleuten *et al.* (2005) reported that increased leptin levels were associated with an increased risk for CVD both in familial combined hyperlipidemia (FCH) patients and in healthy controls, independent of BMI, insulin resistance and gender.

We demonstrated in the present study that serum leptin was an independent factor to determine serum ICAM-1 levels after adjusting for age, sex, BMI, alcohol intake, smoking status, blood lipids such as total cholesterol, triglyceride, HDL cholesterol and LDL cholesterol and HOMA-IR. The study in Mexican Americans which reported the negative correlation between ICAM-1 and leptin is only one that can support our findings (Kent *et al.*, 2004). To our knowledge no *in vitro* or *in vivo* studies have confirmed the direct effects of leptin on cell adhesion molecule expression. Skilton *et al.* (2005) have found that increasing concentrations of leptin did not change either the expression of cellular adhesion molecule (VCAM-1, ICAM-1 and E-selectin) or the degree of monocyte adhesion to endothelial cells. Increasing leptin levels in lean and obese men over 3 d did not alter serum ICAM-1. Moreover, recombinant methionyl human leptin administration in obese subjects with type 2 diabetes mellitus, for 4 or 16 wk, resulting in high pharmacologic leptin levels, did not activate ICAM-1 (Chan *et al.*, 2005). The significant negative relation between leptin and ICAM-1 from our study could not be possibly explained by limited *in vitro* and human administration studies. Therefore, the disagreement in relation between leptin and ICAM-1 is needed to be further investigated.

This study suggests that adiponectin and leptin might be associated with endothelial function. Our results provide an understanding linking obesity, adipokines and endothelial function which lead to atherosclerotic process and cardiovascular disease in hypercholesterolemic patients.

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